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GERMPLASM

Registration of Seven Winter Wheat Germplasm Lines Carrying the *Wsm1* Gene for *Wheat Streak Mosaic Virus* Resistance

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ABSTRACT

Seven winter wheat (*Triticum aestivum* L.) germplasm lines carrying the *Wsm1* gene conferring resistance to *Wheat streak mosaic virus* (WSMV)—Alliance-*Wsm1* (Reg. No. GP-858, PI 653710), Arrowsmith-*Wsm1* (Reg. No. GP-859, PI 653711), Goodstreak-*Wsm1* (Reg. No. GP-860, PI 653712), Harry-*Wsm1* (Reg. No. GP-861, PI 653713), Millennium-*Wsm1* (Reg. No. GP-862, PI 653714), Wahoo-*Wsm1* (Reg. No. GP-863, PI 653715), and Wesley-*Wsm1* (Reg. No. GP-864, PI 653716)—were codeveloped by Washington State University, Pullman, WA; the University of Nebraska, Lincoln, NE; and the USDA-ARS. These seven different winter wheat cultivars were selected to provide more sources of effective resistance to WSMV in winter wheat cultivars of Nebraska and adjoining states. Resistance to WSMV is conferred by the *Wsm1* gene, which was translocated from *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey [*Agropyron intermedium* (Horst.) Beauv.] into wheat. The STSJ15 marker was used to select for the gene in the backcross progeny until the BC_4F_1 generation. In BC_4F_2 generation, screening for disease resistance was done using the Sidney 81 isolate of WSMV, along with the recurrent parents. Lines showing high levels of resistance to WSMV were further selected for seed increase and field evaluation. These lines may serve as a winter wheat source of WSMV resistance and may be used for gene pyramiding and for studying the effect of the *Wsm1* gene in different backgrounds.

Wheat streak mosaic virus (WSMV) belongs to the genus *Tritimovirus* of the Potyviridae family of viruses and is vectored by the wheat curl mite (*Aceria tosichella* Keifer). *Wheat streak mosaic virus* is one of the most common diseases impacting the production of wheat (*Triticum aestivum* L.) worldwide (Brakke, 1971) and is an especially serious pathogen of wheat in the western Great Plains of North America (French and Stenger, 2003). With an average annual yield loss of up to 5%, the disease can be catastrophic (Christian and Willis, 1993; French and Stenger, 2003; Graybosch et al., 2009). For example, Montana's wheat industry alone lost nearly $35 million due to the WSMV outbreak in 1995 (http://www.ars.usda.gov/is/AR/archive/dec00/wheat1200.htm; verified 24 Mar. 2011).

Outbreaks of WSMV are largely due to the presence of volunteer wheat that emerges before harvest and acts as an oversummering host for the build-up of virus-carrying mites, which is further favored by temperatures of 75–80°F (http://pdc.unl.edu/agriculturecrops/wheat/wheatstreakmosaic; verified 24 Mar. 2011). Effective WSMV resistance is very limited in the wheat gene pool. Some germplasm lines, including CO960293–2 (Haley et al., 2002) and the cultivar 'Ron-L' (Seifers et al., 2007), have resistance of unknown origin to WSMV, but that resistance is ineffective at temperatures above 18°C. On the other hand, the resistance gene *Wsm1*, which is transferred from intermediate wheatgrass [*Thinopyrum intermedium* (Host) Barkworth & DR Dewey] (Friebe et al., 1991) to wheat, provides effective resistance at higher temperatures (Seifers et al., 2006; Graybosch et al., 2009). The transfer of resistance to WSMV into wheat cultivars in the United States was done at South Dakota State University by crossing 'Lathrop' spring wheat (Lay et al., 1971) to TA25. TA25 is an octoploid Agrotricum that was

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Abbreviations: MAS, marker-assisted selection; PCR, polymerase chain reaction; STS, sequence-tagged-site; WSMV, *Wheat streak mosaic virus*.

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developed at the Max Plank Institute in Germany from the cross 'Carsten V'/*Agropyron intermedium* (Horst.) Beauv. Further breeding for WSMV resistance led to the development of winter wheat cultivars carrying the *Wsm1* gene with no known negative effects on grain yield (Divis et al., 2006; Graybosch et al., 2009).

Among Nebraskan wheat cultivars where WSMV is the most damaging disease of winter wheat, 'Mace' (Graybosch et al., 2009) is the only cultivar to carry the *Wsm1* resistance gene. To provide more sources of effective resistance to WSMV in winter wheat cultivars of Nebraska and adjoining states, seven different winter wheat cultivars were selected for introgression of the *Wsm1* gene from the donor parent KS93WGRC27 (PI 583794). These include six hard red winter wheat cultivars—'Alliance' (Baenziger et al., 1995), PI 573096; 'Goodstreak' (Baenziger et al., 2004a), PI 632434; 'Harry' (Baenziger et al., 2004b), PI 632435; 'Millennium' (Baenziger et al., 2001), PI 613099; 'Wahoo' (Baenziger et al., 2002), PI 619098; and 'Wesley' (Peterson et al., 2001), PI 605742—along with one hard white winter wheat cultivar—'Arrowsmith' (Graybosch et al., 2005), PI 633911. These seven cultivars are commonly grown in Nebraska and adjoining states. During the 2010 growing season, these accounted for approximately 33% of the total wheat acreage of Nebraska (http://www.nass.usda.gov/Statistics_by_State/Nebraska/Publications/Crop_Variety_Reports/whet2010.pdf; verified 3 May 2011).

Molecular markers linked to genes controlling pest resistance, to difficult-to-screen traits, and to traits of high agronomic values, such as seed-protein content, are now available (Bernardo, 2008). Marker-assisted selection (MAS) is a strategy to capitalize on the available molecular markers and to quickly move valuable traits into elite lines for possible release as cultivars. In this study we have utilized MAS to transfer the *Wsm1* into seven winter wheat cultivars popularly grown in Nebraska and adjoining states.

Methods

The donor of the *Wsm1* gene was a hard red winter wheat line KS93WGRC27 (PI 583794) that carries the gene on a segment from *Agropyron intermedium* (Host) P. Beauv. translocated onto wheat chromosome 4DS (Friebe et al., 1991; Gill et al., 1995). With an objective to reduce the linkage drag, Friebe et al. (2009) were able to recover distal recombinants with 20% of the 4DS-4J^{s}S arm (donor segment). For the present study, the KS93WGRC27 donor line was crossed to each of the hard red winter wheat cultivars: Alliance', 'Goodstreak', 'Harry', 'Millennium', 'Wahoo', and 'Wesley'. A hard white winter wheat cultivar 'Arrowsmith' was also used as one of the recurrent parents. The recurrent parents were always used as females in the crosses. Four backcrosses to the respective recurrent parent were performed for each cross combination. After four backcrosses the plants were selfed to recover homozygosity for the target gene as well as for the rest of the genome. The pedigrees of the germplasm lines containing the *Wsm1* gene are Wahoo*5/KS93WGRC27 (PI 583794); Millennium*5/KS93WGRC27 (PI 583794); Wesley*5/KS93WGRC27 (PI 583794); Alliance*5/KS93WGRC27 (PI 583794); Harry*5/KS93WGRC27 (PI 583794); Goodstreak*5/KS93WGRC27 (PI 583794); and Arrowsmith*5/KS93WGRC27 (PI 583794).

In each backcross, DNA extraction and polymerase chain reaction (PCR) analysis was performed as outlined in Randhawa et al., 2009. Beginning with the BC_1 generation, the sequence-tagged-site (STS) primers STSJ15L and STSJ15R, which are specific for the translocated segment carrying the *Wsm1* gene (Talbert et al., 1996), was used to select the plants carrying the *Wsm1* gene. STSJ15 is a dominant marker amplifying a 400-bp band from the donor parent. No amplification was observed with the STSJ15 primer from any of the seven recurrent parents (Fig. 1a). Additional primers G43L and G43R (G43 markers have no linkage to STSJ15 primers), which amplify a 700-bp fragment from both the donor as well as recurrent parent lines, were used as positive controls for the PCR reactions (http://maswheat.ucdavis.edu/protocols/WSMV/index.htm; verified 24 Mar. 2011) (Fig. 1a). During each backcrossing cycle, plants showing amplification with the STSJ15 primer were selected. At the end of the backcrossing cycles, 5 BC_4F_2 plants showing the STSJ15 amplification band were selfed. Ten plants (BC_4F_3) from the selfed progeny of each of the 5 plants (BC_4F_2) were screened with the marker to identify a BC_4F_2 plant homozygous for the gene (Fig. 1b). The 10 progeny plants from the selected BC_4F_2 plant homozygous for the STSJ15 marker were used for phenotypic screening and seed increase for the germplasm release. The phenotypic screen was performed in a greenhouse at 18°C temperature and light conditions of 16 h day/8 h night. From each line, 10 plants were inoculated with sap from 'Arapaho' wheat infected with the Sidney 81 isolate of WSMV (1:10 weight leaf tissue/volume water) at the two-leaf stage. The wheat variety 'Tomahawk' was

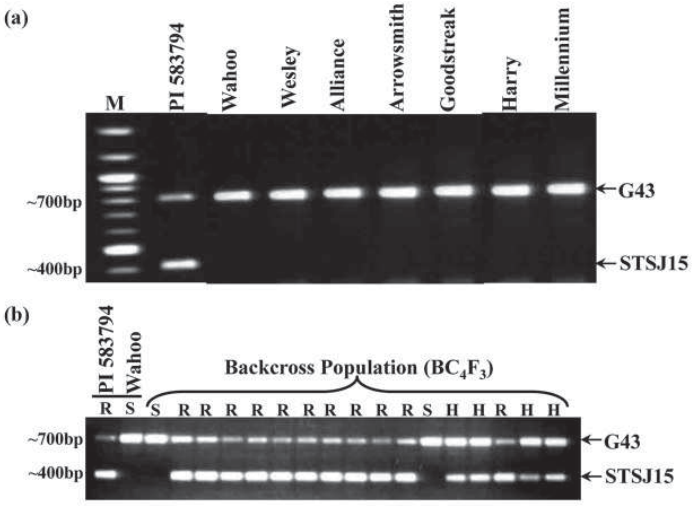

Figure 1. PCR amplification with STSJ15 and G43 primers. (a) Polymorphism survey with STSJ15 primer between donor (PI 583794) and the seven recurrent parents . Primer G43 was used as a control for PCR amplifications. M, size marker. (b) Selection of homozygous BC_4F_2 plant by screening its BC_4F_3 progeny using STSJ15 primer. R, homozygous for the STSJ15 primer; S, absence of STSJ15 primer; H, heterozygous. Arrows indicate the G43- and STSJ15-specific ~700- and ~400-bp amplification product of the polymerase chain reaction.

included as a highly susceptible control. Disease severity (stunting and chlorotic mosaic) was visually rated 4 wk after inoculation using an arbitrary scale of 1 to 6, where 1, 2 = resistant; 3, 4 = moderately susceptible; and 5, 6 = susceptible. A rating of 1 indicates an uninfected plant, and a rating of 6 indicates the same extent of disease as on WSMV-infected Tomahawk.

Table 1. Characteristics of seven winter wheat germplasm lines resistant to *Wheat streak mosaic virus*.†

Line	Donor parent	Disease		Difference in plant height‡	Difference in heading date‡
		Rating	SD		
		1–6§		cm	d
Germplasm					
Alliance-*Wsm1*	PI 583794	1	0.7	–1	+1
Goodstreak-*Wsm1*	PI 583794	1	0.0	0	+1
Harry-*Wsm1*	PI 583794	1	0.3	0	0
Millennium-*Wsm1*	PI 583794	1	0.0	+1	0
Wahoo-*Wsm1*	PI 583794	1	0.0	0	–1
Wesley-*Wsm1*	PI 583794	1	0.6	–2	+2
Arrowsmith-*Wsm1*	PI 583794	2	1.0	+1	+1
Recurrent parent					
Alliance	—	5	—	—	—
Goodstreak	—	6	—	—	—
Harry	—	6	—	—	—
Millennium	—	5	—	—	—
Wahoo	—	6	—	—	—
Wesley	—	6	—	—	—
Arrowsmith	—	6	—	—	—

†Disease rating, plant height, and heading date for germplasm lines were recorded in BC_4F_3 generation. For disease rating 10 plants per line were analyzed; for plant height and heading date, 5 plants per line for each were analyzed.

‡Differences are between lines and recurrent parent. Means for plant height and heading date are not significantly different at P-value < 0.05.

§1–2 = resistant, 3–4 = moderately susceptible, and 5–6 = susceptible.

Characteristics

Agronomic Description

The germplasm lines Alliance-*Wsm1* (Reg. No. GP-858, PI 653710), Goodstreak-*Wsm1* (Reg. No. GP-860, PI 653712), Harry-*Wsm1* (Reg. No. GP-861, PI 653713), Millennium-*Wsm1* (Reg. No. GP-862, PI 653714), Wahoo-*Wsm1* (Reg. No. GP-863, PI 653715), and Wesley-*Wsm1* (Reg. No. GP-864, PI 653716) are hard red winter wheats, whereas Arrowsmith-*Wsm1* (Reg. No. GP-859, PI 653711) is a hard white winter wheat. The phenotypic characteristics of each of these lines were similar to their respective recurrent parents (Table 1). Plant-height and heading-date measurements were recorded and averaged from five plants grown in a greenhouse for both the homozygous germplasm lines as well as for their respective recurrent parents. The height of germplasm lines Wahoo-*Wsm1,* Goodstreak-*Wsm1*, Harry-*Wsm1*, Alliance-*Wsm1,* Millennium-*Wsm1*, and Arrowsmith-*Wsm1* were similar to their respective recurrent parents, whereas Wesley-*Wsm1* was 2.6 cm shorter than its respective recurrent parent (Table 1). Millennium-*Wsm1* and Harry-*Wsm1* headed the same day as their respective recurrent parent. Wahoo-*Wsm1* headed 1 d earlier; Alliance-*Wsm1,* Goodstreak-*Wsm1*, and Arrowsmith-*Wsm1* 1 d later; and Wesley-*Wsm1* headed 2 d later than their respective recurrent parents (Table 1). In summary, the plant heights and heading dates did not show any significant difference between the germplasm lines and their respective recurrent parents (Table 1).

Disease Ratings

The progeny of six germplasm lines (Wahoo-*Wsm1*, Millennium-*Wsm1*, Wesley-*Wsm1*, Alliance-*Wsm1,* Harry-*Wsm1*, and Goodstreak-*Wsm1*) showed a uniform reaction to WSMV with a rating of 1, whereas the progeny of the Arrowsmith-*Wsm1* line showed a uniform rating of 2 (Table 1). Of the seven recurrent parents, Wahoo, Wesley, Harry, Goodstreak, and Arrowsmith showed a very high degree of susceptibility, with a uniform rating of 6. The cultivars Millennium and Alliance showed a low level of tolerance to WSMV and a rating of 5. As illustrated by these results, all seven lines are homozygous for the *Wsm1* gene.

Availability

Twenty grams of seed of each germplasm line has been deposited in the National Small Grains Collection (NSGC), and seed of BC_4F_4 is being grown in Nebraska for field evaluations and seed increase. Small quantities of germplasm seeds can be obtained on request from NSGC and the corresponding author.

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